UNDERSTANDING TRANSMISSION OF SPOREFORMERS IN DAIRY POWDER PRODUCTS

A Thesis

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ABSTRACT

Dairy powder products (e.g., sweet whey, nonfat dry milk, acid whey, and whey protein concentrate-80) are of important economic interest to the dairy industry. According to the U.S. Dairy Export Council, customers have set strict tolerances (<500 to <1,000/g) for thermophilic and mesophilic spores in dairy powders; therefore, understanding proliferation and survival of sporeforming organisms within dairy powder processing plants is necessary to control and reduce sporeformer counts. Raw, work-in process, and finished product samples were collected from four dairy powder processing facilities in the northeast United States over a 1-year period. Two spore treatments were applied: i) 80°C/12 min for sporeformers and ii) 100°C/30 min for highly heat resistant sporeformers. Raw material, work-in-process and finished product samples were analyzed for thermophilic, mesophilic and psychrotolerant sporeformers resulting in 77.5%, 71.0% and 4.6% of samples positive for those organisms, respectively. Work-in-process and finished product samples were analyzed for highly heat resistant thermophilic and mesophilic sporeformers resulting in 63.7% and 42.6% of samples positive, respectively. Results varied considerably by product and plant; sweet whey and non-fat dry milk had the highest overall prevalence of thermophilic and mesophilic sporeformers, whereas acid whey and whey protein concentrate-80 had much lower levels. The results provide a preliminary evaluation of mesophilic and thermophilic sporeformers in various dairy powders. This study also revealed that thermophilic sporeformers are the primary organism of concern in dairy powders and that there must be further study of sporeformers, specifically, thermophilic Sporeformers, within the dairy powder processing continuum, to reduce overall spore counts in finished products.

Key words: thermophilic, mesophilic, sporeformer, milk powder, whey

BIOGRAPHICAL SKETCH

Chief Warrant Officer Four Matthew J. Watterson, Sr. (United States Army) was raised in Homer City, PA and briefly attended Indiana University of Pennsylvania before enlisting in the U.S. Army as a Veterinary Food Inspection Specialist in 1991. During 22 years of service, Matthew developed a keen interest in food safety, auditing, and dairy product processing. He has completed numerous Food & Drug Administration food safety courses and has earned American Society for Quality credentials as a Certified Quality Auditor and Certified Hazard Analysis Critical Control Point (HACCP) Auditor. Matthew has served at numerous duty locations in both the United States and abroad, including Fort Knox, KY; El Gorah, Egypt; Fort Sam Houston, TX; Fort Irwin CA; Fort Lewis, WA; and Camp Arifjan, Kuwait (deployed in support of *Operations Iraqi and Enduring Freedom* – 2007). More recently, Matthew served as the Senior Food Protection Officer for the Japan District Veterinary Command (Camp Zama, Japan). He served as the key food safety advisor in the unit that was responsible for food and water safety and public health for more than 130,000 United States citizens (Soldiers and civilians) living in Japan. Matthew advised the command on food and water safety following the Great Tohoku Earthquake of 2011, the associated tsunami, and the Fukushima nuclear power plant disaster.

While in service, Matthew has been recognized with numerous medals of commendation for meritorious achievement and service. He holds an Associate in Science degree from the University of the State of New York, Regents College (1995) and a Bachelor of Science degree in Food Science and Industry from Kansas State University (2011). Matthew's professional memberships include the United States Army Warrant Officers Association; the Association of the United States Army; the Institute of Food Technologists; the American Society for Quality, and the International Association for Food Protection.

Matthew and his wife, Cynthia (Sergeant First Class, US Army – Retired), have three children, Christopher, Shemaira and Matthew, Jr. The family currently resides in Ithaca, NY and will move to Fort Hood, TX during the summer of 2013.

Dedicated to my loving, patient,
and very supportive family:

Mrs. Cynthia Watterson
Mr. Christopher M.J. Watterson
Ms. Shemaira Y. Watterson
Master Matthew J. Watterson, Jr.

and in everlasting memory of my beloved father:

Mr. Jarvie J. Watterson
1948 – 2013

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[1]The views and opinions expressed in this document are those of the author and in no way represent the official policy or position of the United States Army Medical Department, the United States Army, the Department of Defense or the United States Government.

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LIST OF ABBREVIATIONS

AWP	Acid Whey Powder
B	Beginning
BHI	Brain Heart Infusion (agar)
cfu	Colony Forming Unit
E	End
FP	Finished Product
g	gram
HTST	High Temperature Short Time
HHR	Highly Heat Resistant
ISO	*International Organization for Standardization*
LE	Lab Error
MSC	Mesophilic Spore Count
M	Middle
MQIP	*Milk Quality Improvement Program*
mL	milliliter
NFDM	Nonfat Dry Milk
NA	Not Applicable
NS	No Sample
NT	Not Tested
PSC	Psychrotolerant Spore Count
R	Raw
RO	Reverse Osmosis
SFs	Sporeformers
SMEDP	*Standard Methods for the Examination of Dairy Products*

LIST OF ABBREVIATIONS (continued)

SMP	Skim Milk Powder
SP	Spore Pasteurized
STR	Specially [*sic*] Thermally Resistant
SWP	Sweet Whey Powder
TSC	Thermophilic Spore Count
UF	Ultra Filtration
UHT	Ultra High Temperature
USDEC	*United States Dairy Export Council*
WPC-80	Whey Protein Concentrate-80
WMP	Whole Milk Powder
WIP	Work-In-Process

INTRODUCTION

Sporeforming organisms have been detected throughout the dairy processing continuum (Crielly et al., 1994; Postollec et al., 2012), including dairy farm environments, storage and transportation tanks, and in dairy processing plants. On the dairy farm, sporeforming organisms have been isolated from soil and teats (Christiansson et al., 1999); pasture (Slaghuis et al., 1997); bedding, silage, and feed (Crielly et al., 1994; te Giffel et al., 2002; Magnusson et al., 2007); fecal material (Labots et al., 1965; Huck et al., 2008) and in raw milk (Boor et al., 1998; Huck et al., 2007; Martin et al., 2011). During intermediate storage and transportation, sporeforming organisms have been detected in bulk tank raw milk (Griffiths and Phillips, 1990; Crielly et al., 1994) and in raw milk in transport tankers (Huck et al., 2007). Sporeformers (**SFs**) have been isolated from the environment of dairy product processing plants (Ralyea et al., 1998; Fromm and Boor, 2004; Huck et al., 2007) and have been detected in ready-to-eat pasteurized dairy products, e.g., fluid milk (Fromm and Boor, 2004; Huck et. al., 2007); in Gouda and semi-hard cheeses (Klijn et al., 1995); and in dairy powder products (Murphy et al., 1999; Ronimus et al., 2003; Scott et al., 2007; Burgess et al., 2009; Burgess et al., 2010).

Aerobic SFs of particular concern in dairy products include the psychrotolerant *Paenibacillus* spp. (Fromm and Boor, 2004; Huck et al., 2007; Ranieri et al., 2009; Ivy, et al., 2012), mesophilic *Bacillus* spp., e.g., *B. licheniformis*, *B. subtilis*, and *B. pumilus*, (Crielly et al., 1994; Fromm and Boor, 2004) and thermophilic *Anoxybacillus flavithermus* and *Geobacillus* (Burgess et al., 2009). The psychrotolerant SFs, such as *Paenibacillus* spp., are known to reduce shelf-life and cause spoilage of fluid milk products (Fromm and Boor, 2004; Durak et al., 2006; Huck et al., 2007; Ranieri et al., 2009). Mesophiles can cause shelf-life or keeping quality problems in shelf-stable milk products (Ridgway, 1954; Ridgway, 1955; Franklin, et al., 1956). Thermophiles, such as *A. flavithermus* and *Geobacillus* spp. are known to cause quality and shelf-life concerns in products that are manufactured using dairy powders as ingredients (Muir et al., 1986; Flint et al., 1997). Other members of the *Bacillus* genus have also been known to cause

quality defects in ultra-high temperature (**UHT**) milk and retorted products (Klijn et al., 1997; Scheldeman et al., 2006). *Clostridium tyrobutyricum*, a sporeformer and obligate anaerobe, is known to cause late-blowing defects in Gouda and other cheeses during aging (Klijn et al., 1995; Quiberoni et al., 2008). Quality and defects that arise during the shelf-life of dairy products result in food waste and create economic losses for dairy product manufacturers.

Sporeforming bacteria can survive the conditions found in food processing facilities due to their innate ability to resist adverse conditions by entering a resilient, dormant state, through the formation of endospores. Spores ensure survival of the organism, as they have the ability to withstand arduous environmental conditions, including: reduced nutrient availability; pH extremes; adverse temperatures; and reduced moisture conditions (De Vos, 2009). Within food production environments, spores survive the heating temperatures used to cook food products or to pasteurize milk (Collins, 1981), and resist the chemicals used to clean and sanitize equipment, surfaces, and utensils (Russell, 1990; Bloomfield and Arthur, 1994). When the environment returns to favorable conditions, the endospores activate, germinate, and return to the vegetative cell state through outgrowth, which is then followed by reproduction (De Vos, 2009).

As an additional survival mechanism, many bacteria – including sporeformers – can form biofilms, which further enhances the bacteria's ability to persist under adverse environmental conditions (Zottola and Sasahara, 1994; Bower et al., 1996). Biofilm formation can be particularly problematic in food processing (Zottola and Sasahara, 1994; Kumar and Anand, 1998) and dairy processing environments (Flint et al., 1997; Faille et al., 2001; Burgess et al., 2009), as biofilms provide protection to vegetative cells, and their spores, from the biocidal effects of cleaning and sanitizing agents (Hood and Zottola, 1995; Kumar and Anand, 1998).

Vegetative cells and spores of sporeforming organisms (e.g., *Geobacillus*, *Anoxybacillus*, *B. licheniformis*, *B. coagulans* and *B. pumilis*) are of particular concern to the dairy industry because *Bacillus* spp. have been shown to adhere strongly to stainless steel (Flint, et al., 1997; Parkar et al., 2001; Palmer et al., 2010), which is commonly found in dairy processing facilities, and to stainless steel with milk foulant (Flint et al., 2001a). Strong adhesion of the bacterial cells

and spores to stainless steel surfaces further enhances biofilm development (Zottola and Sasahara, 1994; Kumar and Anand, 1998). Spores and vegetative cells that have become attached in biofilms may eventually detach and contaminate equipment downstream and finished product (Hood and Zottola, 1995; Bower et al., 1996; Flint et al., 1997; Burgess et al., 2009) which can result in reduced shelf-life and increased quality defects in manufactured dairy products (Wong et al., 1988; Marchand et al., 2012).

During a previous study, Murphy et al. (1999) isolated *B. stearothermophilus* (now, *Geobacillus*) and *B. licheniformis* from the tubular preheater(s) and the evaporator in a milk powder processing facility. More recently, Scott et al. (2007) isolated *A. flavithermus* and *Geobacillus* from the preheater (plate heat exchanger) and from the evaporator in a milk powder processing plant. It is widely held within the dairy processing industry that sporeformer counts in dairy product powders increase as the production run increases, whereby longer runs produce higher counts. Murphy et al. (1999) reported increased growth in the evaporators within 4 hours, and significant contamination of the evaporator system after 8 hours. Scott et al. (2007) found that sporeformer counts increased at both the preheater and evaporator steps between hours 9 and 18 of a whole milk powder processing run. These increases have been attributed to the contamination of the product from biofilm build-up and the sloughing-off of foulant.

Recently, customers have set strict specifications for mesophilic and thermophilic sporeformers. For example, at the United States Dairy Industry Spore Seminar (San Francisco, February 2013), a representative of the United States Dairy Export Council (**USDEC**) presented sporeformer specifications from international customers that delineate the following spore count limits for dairy powders: aerobic mesophilic and thermophilic spore count <500 to <1000 cfu/g for skim milk powder (**SMP**), non-fat dry milk (**NFDM**) and whole milk powder (**WMP**) destined for infant formula; and <500 to <2000 cfu/g for aerobic thermophilic spores in SMP and WMP destined for recombined or UHT products (Bienvenue, 2013). These stringent sporeformer specifications are very difficult to achieve and present an important challenge to the dairy industry, worldwide.

Reduction of sporeformer counts in finished dairy powder products to meet strict customer specifications requires a systematic approach to understanding the sources and niches that contribute to contamination of milk products with sporeforming organisms, from the farm, through distribution, and during processing. To this end, we surveyed four US dairy powder products, over a 12-month period, to determine the prevalence and in-plant contamination patterns for psychrotolerant, mesophilic, and thermophilic SFs.

MATERIALS AND METHODS

Dairy Powder Processing Plants. Four dairy powder product processing facilities (plants A, B, C and D, Table 1), located in the northeastern United States, voluntarily participated in the yearlong study. Each of the four plants manufactures one of the following dairy powder products; i) sweet whey powder (**SWP**; Plant A); ii) nonfat dry milk (**NFDM**; Plant B); iii) acid whey (**AW**; Plant C); and iv) whey protein concentrate-80 (**WPC-80**; Plant D). The NFDM plant used previously untreated raw milk as the sole raw ingredient; the three whey powder plants utilized whey by-products, which resulted from cheese manufacturing operations, as raw ingredient(s). Within the four processing facilities, production runs varied between 10-12 hours for the shortest runs and 18-24 hours for the longest.

Table 1. Summary of plant characteristics and sample points for four northeast dairy powder processing facilities

Plant	Product	# Sample Points (Raw)[1]
A	Sweet Whey	9 (4)
B	Nonfat Dry Milk	6 (1)
C	Acid Whey	4 (1)
D	Whey Protein Concentrate-80	7 (1)

[1]Number of sample points where raw ingredients were collected are indicated within parentheses

Sampling Scheme and Collection. During the twelve-month sampling period, raw ingredients (**R**), work-in-process (**WIP**) and finished product (**FP**) samples were aseptically collected once every two months from multiple sample points within each plant. Depending on processing parameters, or individual plant design and set-up, samples were collected at four, six, seven, or nine sampling points along the production line (Table 1; Figure 1). Sample collection occurred at three time points, beginning (**B**; collected within two hours of the processing run start-up), middle (**M**; collected within two hours of the projected midpoint of the run), and end (**E**; collected within the last two hours of production).

Raw ingredients collected at each time point (B, M, and E) included; i) raw milk; ii) whey by-products from cheese making (e.g., cheddar, ricotta, mozzarella, and cottage cheese); iii) lactose (powder); iv) WPC-80 (powder); and v) lecithin (liquid). Raw fluid samples were aseptically collected in 500 mL sterile Whirl-Pak™ bags from storage tanks or silos (Figure 1). Dry, raw ingredient samples were collected in 100 g portions and were deposited into sterile Whirl-Pak™ bags, using sterile scoops, from aseptically opened bulk ingredient bags (only from those plants that used dry raw ingredients within the process).

WIP samples (fluid, 500 mL or powder, 100 g) were aseptically collected, in sterile Whirl-Pak™ bags, from intermediate locations along the production line at each time point (B, M, and E). WIP sample points included; i) balance tank(s); ii) intermediate storage tank(s); iii) post-ultrafiltration or reverse osmosis concentration; iv) post-evaporator; v) condensed product silos and; vi) processing steps after the spray drier (e.g., at the sifter, slide or belt) (Figure 1). WIP samples were collected from intermediate holding tanks using washed and sanitized petcocks or valves, or sanitized dipping devices when necessary, depending on sample point location and plant configuration. When samples were collected using a petcock or valve, the

device was chemically sanitized (e.g., with iodine or calcium hypochlorite), and the petcock or valve was flushed for 10-15 seconds to expel one-half to one gallon of fluid product – in accordance with *Standard Methods for the Examination of Dairy Products*, 17th Edition (***SMEDP***; Graham, 2004). Dry WIP samples were collected aseptically from conveyor belts using sterile scoops or from falling powders (e.g., at the slide after the spray-drier, or from the sifter [Figure 1]), using suspended, sterile Whirl-Pak™ bags.

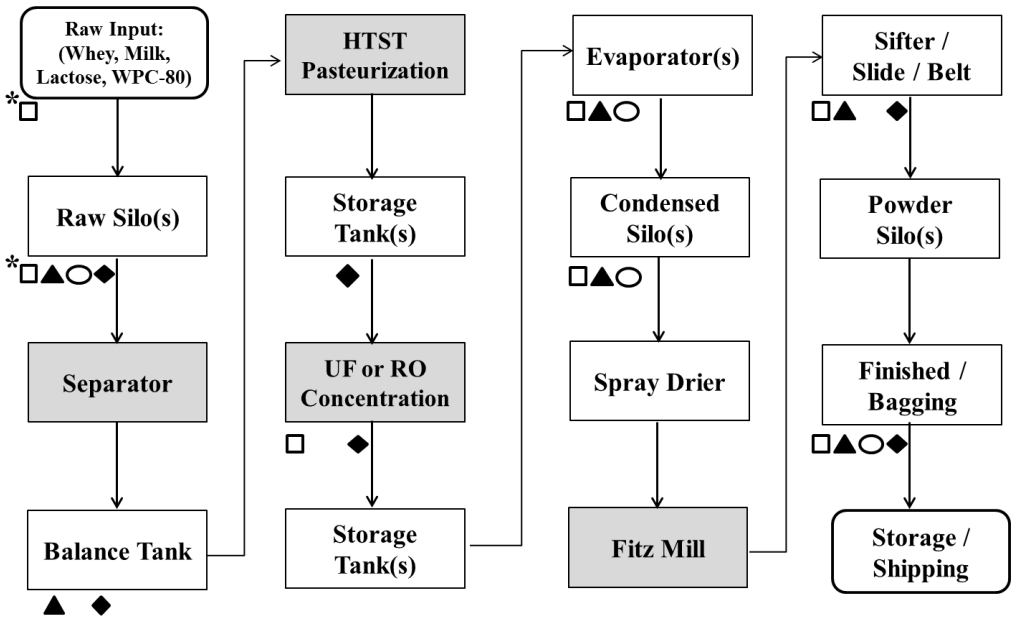

Figure 1. Consolidated flow diagram representing basic processing steps for all four processing plants; shaded boxes represent process steps that are not used in every plant process. Actual sample points, which varied between 4 and 9 sample points per plant, are represented by symbols: □ = sweet whey; ▲= nonfat dry milk; ○ = acid whey; and ◆ = whey protein concentrate-80. Sample points for select raw ingredients (e.g., WPC-80, lactose and lecithin), or where there may be more than one raw ingredient tank, are marked with an * (only applicable to select processes).

HTST = High temperature, short time; UF = ultra-filtration; and RO = reverse osmosis.

Finished product samples were aseptically collected using sterile scoops (Figure 1) from unsealed or aseptically opened FP bags. All samples were held at or below 6°C awaiting transport to the Milk Quality Improvement Program (**MQIP**) laboratory (Cornell University; Ithaca, NY). Samples were packed on ice in insulated coolers and were shipped by overnight delivery or were picked up at the processing plant by MQIP laboratory personnel within 24h of sampling. Temperature controls were included in each cooler and were evaluated immediately upon sample arrival at the laboratory; samples with temperatures >6°C were rejected and replacement samples were collected later.

Spore Count Treatments, Plating and Enrichments. A spore pasteurization (**SP**) treatment was performed on all samples in accordance with *SMEDP* (Frank and Yousef, 2004) for enumeration of aerobic bacterial spores. Dry WIP and FP samples were rehydrated in phosphate buffer solution with magnesium chloride (Weber Scientific, Hamilton, NJ) at a ratio of 1:10 w/w. Fluid product and rehydrated powder samples (100 mL) were placed in sterile, glass Pyrex® (Germany) bottles, heated to 80°C for 12 min to inactivate all vegetative cells, and rapidly cooled to <6°C in an ice bath. All samples were spiral plated (Spiral Biotech Autoplate® 4000, Norwood, MA), in duplicate, on Brain Heart Infusion (**BHI**) Agar for each of the following tests: i) Psychrotolerant Spore Count (**PSC**; incubated at 6°C for 10d); ii) Mesophilic Spore Count (**MSC**; incubated at 32°C for 48h); and iii) Thermophilic Spore Count (**TSC**; incubated at 55°C for 48h). Additional dilution of certain samples was necessary, post-heat treatment, prior to spiral plating. Colonies were enumerated immediately following each incubation period.

Additionally, immediately following the spore treatment and subsequent cooling, 30 mL aliquots of each spore treatment were placed in three separate 60 mL vials to allow for

enrichment that enabled detection of lower levels of psychrotolerant, mesophilic and thermophilic SFs. Enrichment vials were incubated under the following conditions: i) PSC, 6°C for 10d; ii) MSC, 32°C for 48h; and iii) TSC, 55°C for 48h. For those samples where the final count on direct plating was below the detection limit (i.e., no colonies present on BHI; Supplemental Table S1), a 10 µL aliquot of the enriched sample was streaked onto BHI and incubated as follows: i) PSC-enrichment, 6°C for 10d; ii) MSC-enrichment, 32°C for 48h; and iii) TSC-enrichment, 55°C for 48h (MSC and TSC enrichment plates were checked at 24h for colony formation). After the incubation period, streaked enrichment plates were examined for presence or absence of visible colonies.

Evaluation of WIP and FP samples for highly heat resistant (**HHR**) spores was performed by heating both fluid and rehydrated powder (1:10 w/w in phosphate buffer) samples to 100°C for 30 minutes in a boiling water bath, then rapidly cooling to <6°C prior to being spiral plated, in duplicate, on BHI. In the same manner as the spore treated samples, HHR treated samples were tested for i) MSC and ii) TSC at the same corresponding incubation parameters listed above. Additional dilution of certain samples was necessary post-heat treatment prior to spiral plating. After incubation, visible colonies were enumerated immediately, as described above.

As with the spore treated samples, 30mL aliquots of HHR treated samples were incubated at MSC and TSC parameters in individual 60mL sterile vials to allow for the enrichment of lower levels of highly heat resistant mesophilic and thermophilic SFs. A 10 µL aliquot of the enriched sample was streaked onto BHI if the direct plated test (HHR MSC or HHR TSC) was below the detection limit (i.e., no colonies present on direct plating; Supplemental Table S1). Enrichment plates were incubated at the corresponding MSC and TSC incubation parameters listed above and evaluated for presence or absence of visible colonies.

Isolate Preservation. For each sample that displayed growth, on direct plating or after plating of enrichments, bacterial isolates were collected from BHI plates representing each test type (PSC, MSC, TSC, HHR MSC, and HHR TSC). Colonies representing each visually distinct morphology were selected and pure cultures were streaked for isolation. Single colonies were picked from the plates and streaked onto BHI agar. After 18-24h incubation (at 32°C for PSC, MSC and HHR MSC, or 55°C for TSC and HHR TSC), single colonies were selected from the pure culture and inoculated into BHI broth. The samples in broth were incubated, at the temperatures described above for 12-18h. Aliquots were selected and isolates were preserved in a 15% glycerol solution and then stored in duplicate at minus 80°C. Further information on isolates collected in this study (e.g., FSL W8-0001, FSL W8-0002…) can be found at www.foodmicrobetracker.net.

Statistical Analysis. Statistical analyses were performed in JMP (Version 10.0, SAS Institute Inc., Cary, NC). Microbiological data were log-transformed prior to calculating means and both linear and logistic regressions were performed.

RESULTS AND DISCUSSION

Thermophilic Spores Appear to Dominate Spore Populations in Dairy Powders. Overall, 4.6%, 71.0% and 77.5% of samples were positive for psychrotolerant, mesophilic or thermophilic spores, either by direct plating or enrichment. Utilizing the methods described, positive on direct plating indicates >10 cfu/ml or 100 cfu/g, and positive for enriched samples indicates >0.03 cfu/mL, unless additional dilution of samples prior to plating was required (Supplemental Table S1). PSC spore treated samples from all four plants, resulted in 1.7% of samples positive after direct plating and an additional 2.9% positive after enrichment (Supplemental Table S1). The vast majority of samples (95.4%) had no evidence of psychrotolerant SFs, even after enrichment for low levels of these organisms (Supplemental Table S1). This result is not surprising, as the high temperatures of powder processing, typically in the 40-80°C range, should select for organisms that prefer to grow at those temperatures. These results also imply that the likelihood of quality defects resulting from psychrotolerant SFs in finished powder products is relatively low. It should be noted, however, that despite the low incidence of psychrotolerant SFs found in dairy powders in this study, previous work has demonstrated that extremely low levels of psychrotolerant sporeforming bacteria present in refrigerated fluid milk can result in quality defects and reduced shelf-life (Ranieri et al., 2012); two issues which remain a concern for fluid milk processors.

In contrast to results for psychrotolerant SFs in dairy powders, 37.8% of all SP treated samples were positive after direct plating for mesophilic SFs; 33.2% were positive after enrichment and 29.0% had no detectable mesophilic SFs (Table 2). The organisms likely to represent the majority of the mesophilic ecology found in these dairy powders are the spores of

B. licheniformis, B. subtilis and *B. pumilus* which have been isolated from previous dairy powder processing studies (Burgess et al., 2010; Yuan et al., 2012).

Finally, our results indicate that 59.4% of spore treated samples had thermophilic SFs – at levels high enough to be detected on direct plating – and 18.1% of samples were positive for TSC after enrichment; only 22.5% of the samples had no detectable thermophilic SFs (Table 2). Organisms generally characterized as thermophilic SFs include *A. flavithermus* and *Geobacillus* spp. (Scott et al., 2007); these organisms grow optimally at temperatures of 40 to 65°C (Flint et al., 2001b; Parkar et al., 2003; Scott et al, 2007). Murphy et al. (1999), Scott et al. (2007), and Seale et al. (2008 and 2012) have previously isolated these organisms from preheaters (tubular and plate) and evaporator(s) in dairy processing powder environments; therefore, the prevalence of these organisms in our samples, at 77.5%, indicate that thermophilic SFs may be the primary organisms of concern in dairy powder products.

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Table 2. Summary of thermophilic & mesophilic spore counts in dairy powders from 4 northeast dairies sampled 6 times over a 12-month period

			Thermophilic Spore Count			Mesophilic Spore Count		
Plant (Product)	**Product Status[1]**	**Time Point (no. of samples)[2]**	**% Samples Positive After Direct Plating (mean log cfu/mL or g)[3]**	**% Samples Positive After Enrichment**	**% Samples Negative**	**% Samples Positive After Direct Plating (mean log cfu/mL or g)[3,4]**	**% Samples Positive After Enrichment**	**% Samples Negative**
A (Sweet Whey)	R	B (18)	78 (2.7)	6	16	44 (1.6)	17	39
		M (12)	83 (2.9)	17	0	17 (1.0)	42	41
		E (11)	91 (2.6)	9	0	9 (1.0)	55	36
	WIP	B (24)	100 (3.3)	0	0	33 (1.9)	33	34
		M (24)	100 (3.3)	0	0	21 (1.4)	50	29
		E (23)	100 (3.4)	0	0	39 (2.1)	52	9
	F	B (6)	100 (4.2)	0	0	17 (2.0)	67	16
		M (6)	100 (4.2)	0	0	17 (2.0)	83	0
		E (6)	100 (4.3)	0	0	33 (2.0)	67	0
	Total / Mean[5]	130	94.6 (3.3)	3.1	2.3	28.5 (1.8)	45.4	26.1
B (NFDM)	R	B (6)	83 (1.6)	17	0	80 (1.6)	20	0
		M (6)	67 (1.3)	20	13	100 (1.6)	0	0
		E (6)	67 (1.5)	20	13	50 (1.4)	50	0
	WIP	B (24)	79 (2.1)	21	0	83 (1.9)	17	0
		M (24)	92 (2.2)	8	0	88 (1.9)	12	0
		E (22)	100 (3.2)	0	0	86 (2.0)	14	0
	F	B (6)	100 (2.6)	0	0	100 (2.1)	0	0
		M (6)	83 (2.3)	17	0	83 (2.2)	17	0
		E (6)	100 (3.3)	0	0	100 (2.3)	0	0
	Total / Mean[5]	106	87.7 (2.4)	10.4	1.9	85.7 (1.9)	14.3	0.0
C (Acid Whey)	R	B (6)	17 (1.0)	0	83	NA	0	100
		M (6)	NA	0	100	17 (1.3)	17	66
		E (6)	NA	0	100	NA	0	100
	WIP	B (12)	8 (1.0)	8	84	25 (1.5)	17	58
		M (12)	33 (1.6)	8	59	42 (1.2)	33	25
		E (12)	25 (1.5)	0	75	42 (1.4)	8	50
	F	B (6)	33 (2.3)	0	67	NA	33	67
		M (6)	17 (1.5)	0	83	33 (1.7)	0	67
		E (6)	17 (1.0)	0	83	NA	0	100
	Total / Mean[5]	72	18.1 (1.5)	2.8	79.1	22.2 (1.4)	13.9	63.9
D (WPC-80)	R	B (8)	25 (1.0)	0	75	13 (1.7)	13	74
		M (6)	33 (1.2)	17	50	NA	17	83
		E (6)	NA	17	83	33 (1.2)	50	17
	WIP	B (23)	22 (1.9)	52	26	9 (1.7)	61	30
		M (23)	13 (2.0)	65	22	9 (2.2)	45	46
		E (24)	8 (2.5)	67	25	17 (2.2)	42	41
	F	B (5)	NA	100	0	20 (2.6)	80	0
		M (5)	20 (2.3)	80	0	20 (2.3)	80	0
		E (6)	33 (2.5)	67	0	NA	100	0
	Total / Mean[5]	106	16.0 (1.9)	54.7	29.3	12.3 (2.0)	50.0	37.7
TOTAL	**AVERAGE**	**414**	**59.4 (2.8)**	**18.1**	**22.5**	**37.8 (1.8)**	**33.2**	**29.0**

[1]R = Raw; WIP=Work in process; F=Finished product

[2]B=Beginning (sampled within 2h of start of run); M=Middle (sampled w/in 2h of midpoint of run); E=End (sampled w/in last 2h of run)

[3]NA = Not Applicable

[4]Sample Size (n) for Plant B, Raw, MSC was five (5)

[5]Refers to total samples per plant; mean percentage of samples positive and (mean log cfu/mL or g)

Incidence of Highly Heat Resistant Spores is Considerably Lower than Other Spores in Dairy Powders. Results of the HHR spore treatment indicate that only 7.3% of all WIP and finished product samples tested were positive on direct plating for HHR mesophilic SFs and an additional 35.3% were positive after enrichment (Table 3). As a result, 57.4% of samples treated for highly heat resistant spores had no detectable mesophilic SFs (Table 3). In contrast, 41.7% and 35.7% of SP treated WIP and FP samples were positive on direct plating, respectively, and an additional 33.6% and 42.9% of WIP and FP samples were positive for mesophilic sporeforming bacteria after enrichment (Supplemental Table S1).

The results for HHR are not unexpected given the severity of the HHR spore treatment, which is designed to select primarily for obligate thermophiles (Burgess et al., 2010). After plating, 32.8% of samples tested were positive for HHR thermophilic SFs; 30.9% were positive following enrichment; and the remaining 36.3% had no detectable HHR thermophilic SFs (Table 3). Interestingly, 59.8% and 60.0% of SP treated WIP and FP samples, respectively, were positive for thermophilic sporeforming bacteria on direct plating; and an additional 22.0% and 20.0% of WIP and FP samples were positive for thermophilic sporeforming bacteria after enrichment (Supplemental Table S1). These data reinforce our position that thermophilic SFs are the primary organisms of concern in dairy powder products.

Dairy powders are used in a wide range of products including bakery items, infant products, processed meats and seafood, beverages, pet foods, canned foods and UHT products. There are three heat treatments that are typically used to enumerate spores in dairy powders, of which, we applied two in this study: i) 80°C for 12m for sporeformers, and ii) 100°C for 30m for HHR spores. Some researchers have suggested that HHR spores may be isolated using a method described by ISO/TS 27265:2009, which applies a spore heat treatment of 106°C / 30m and an

Table 3. Summary of highly heat resistant thermophilic & mesophilic spore counts in dairy powders from 4 northeast dairies sampled 6 times over a 12 month period

			Highly Heat Resistant Thermophilic Spore Count			Highly Heat Resistant Mesophilic Spore Count		
Plant (Product)	**Product Status[1,2]**	**Time Point (no. of samples)[3]**	**% Samples Positive After Direct Plating (mean log cfu/mL or g)[4]**	**% Samples Positive After Enrichment**	**% Samples Negative**	**% Samples Positive After Direct Plating (mean log cfu/mL or g)[4]**	**% Samples Positive After Enrichment**	**% Samples Negative**
A (Sweet Whey)	WIP	B (24)	54 (2.3)	8	38	17 (1.3)	38	45
		M (24)	67 (2.1)	8	25	21 (1.1)	33	46
		E (23)	61 (2.5)	9	30	4 (1.3)	39	57
	F	B (6)	33 (3.3)	50	17	17 (2.0)	33	50
		M (6)	67 (3.2)	17	16	NA	50	50
		E (6)	83 (3.1)	0	17	17 (2.5)	17	66
	Total / Mean[5]	n = 89	60.7 (2.5)	11.2	28.1	13.5 (1.4)	36.0	50.5
B (NFDM)	WIP	B (24)	33 (1.8)	63	4	21 (2.1)	42	37
		M (24)	42 (2.3)	54	4	4 (1.3)	79	17
		E (22)	82 (2.5)	18	0	5 (1.3)	82	13
	F	B (6)	67 (2.0)	33	0	NA	83	17
		M (6)	33 (1.9)	67	0	NA	67	33
		E (6)	100 (3.1)	0	0	NA	67	33
	Total / Mean[5]	n = 88	54.5 (2.3)	43.2	2.3	8.0 (1.9)	68.2	23.8
C (Acid Whey)	WIP	B (12)	NA	0	100	NA	8	92
		M (12)	NA	17	83	NA	0	100
		E (12)	NA	0	100	NA	8	92
	F	B (6)	NA	0	100	NA	0	100
		M (6)	NA	0	100	NA	0	100
		E (6)	NA	0	100	NA	17	83
	Total / Mean[5]	n = 54	NA	3.7	96.3	NA	5.6	94.4
D (WPC-80)	WIP	B (23)	4 (2.6)	43	53	4 (1.0)	22	74
		M (23)	4 (2.3)	52	44	4 (2.1)	13	83
		E (24)	NA	46	54	8 (3.4)	25	67
	F	B (5)	NA	100	0	NA	20	80
		M (5)	NA	100	0	NA	20	80
		E (6)	NA	83	17	NA	17	83
	Total / Mean[5]	n = 86	2.3 (2.5)	55.8	41.9	4.7	19.8	75.5
TOTAL	**AVERAGE**	**n = 317**	**32.8 (2.4)**	**30.9**	**36.3**	**7.3**	**35.3**	**57.4**

[1]Raw materials were not analyzed for highly heat resistant spores
[2]WIP=Work in process; F=Finished product
[3]B=Beginning (sampled within 2h of start of run); M=Middle (sampled within 2h of midpoint of run); E=End (sampled within last 2h of run)
[4]NA= Not Applicable
[5]Refers to total samples per plant; mean percentage of samples positive and (mean log cfu/mL or g)

incubation temperature of 55°C. This method selects for the "specially [*sic*] thermoresistant spores" (**STR**), of *Anoxybacillus* spp. and *Geobacillus* spp. that are known to cause defects in canned and UHT products. In discussions with dairy powder producers, we have become concerned that improper application of the STR method may exclude the spores of some *Bacillus* spp., especially those organisms of the genus *Bacillus* that are mesophilic, can survive high heat treatment, and can then grow at thermophilic temperatures (e.g., *B. subtilis*, *B. pumilus* and *B.*

licheniformis). Appropriately, the STR method specifically selects for thermophilic organisms that would be of primary concern in dairy powders destined for UHT or retorted products, as described by Burgess et al. (2010). Use of the STR method may offer a more accurate depiction of the key organisms of concern in some finished products; we feel that the STR method should not be used exclusively for all HHR sporeformers.

It is important that comparisons of spore counts in finished dairy powder products are made carefully, especially due to the variations in the two methods used to enumerate HHR spores. Therefore, caution must be applied, when specifications are set and laboratory procedures are selected, to ensure that mesophiles that grow at thermophilic temperatures are not inadvertently or unintentionally inactivated by selection of a specific laboratory procedure. The apparent lack of standardization, in the selection and application of these methods throughout the dairy powder industry, underscores the importance for both manufacturers and customers to understand the available laboratory methods used to enumerate sporeforming organisms and the proper application thereof.

Spore Load in Dairy Powders Varies Widely Based on Products. The dairy powders tested in the current study included SWP, NFDM, AW and WPC-80, all of which have very different product characteristics and undergo different processing conditions. It is therefore not surprising that our results indicate that spore loads in these four products vary considerably. Our results show that 94.6% and 87.7% of SWP and NFDM, respectively, were positive for TSC after direct plating (Table 2). Conversely, only 18.1% and 16.0% of AWP and WPC-80, respectively, were positive for TSC after direct plating (Table 2). A similar trend was identified for MSC with 28.5% and 85.7% of SWP and NFDM, respectively, positive by direct plating, and 22.2% and

12.3% of AWP and WPC-80 positive, respectively (Table 2). These data suggest that, generally, SWP and NFDM have higher spore loads than AWP and WPC-80.

Additionally, HHR TSC spore data show a supporting pattern, with 60.7% and 54.5% of SWP and NFDM, respectively, positive following direct plating. Conversely, no AWP samples and only 2.3% of WPC-80 samples were positive following direct plating (Table 3). The SFs for which the HHR treatment selects (i.e., *A. flavithermus* and *Geobacillus* spp.) are more likely to grow at the incubation temperature used in the TSC test (55°C) as opposed to those utilized by the MSC test (32°C). Therefore, not surprisingly, all powder products subjected to the HHR treatment had lower incidence of HHR MSC, ranging from a low of 0% positive after direct plating in AWP to a high of 13.5% positive after direct plating in SWP (Table 3).

Spore Counts Increase from Raw to Finished in the Dairy Powder Product Processing Continuum but not Throughout a Processing Run. As expected, our results from SWP and NFDM indicate that the SP and HHR spore counts increase as the materiel moves from raw product through the finished product continuum (Figures 2 and 3; data for AWP and WPC-80 not shown due to low incidence of SFs). This increase in spore counts may be attributed to at least two mechanisms that must be considered: i) physical concentration of the product (liquid to powder) and ii) the introduction of additional microorganisms not present in the raw material acquired from the processing environment or conditions therein (e.g., through biofilm slough-off or environmental contamination). The contribution of the first mechanism, the physical concentration of fluid milk or liquid whey into finished powder, is easily evaluated, in theory, by examining the concentration factor for these two products. Considering that 100g of skim milk results in 10g of NFDM and 100g of sweet whey results in 6g of SWP and applying the raw to

finished product concentration factor to the SWP data in our study, we would expect, for example, that the raw material with a mean TSC of 2.7 log cfu/mL (Figure 2) would result in finished product with a mean TSC of 3.9 log cfu/g (Figure 2) if the only contributing factor to the finished product count was the physical concentration of the raw material. Based on the actual finished product mean TSC of 4.2 log cfu/g at the B timepoint (Figure 2), it appears that there may also be a contribution from microorganisms entering the product throughout the processing continuum (e.g., the processing plant environment). Further, at the M and E timepoints, we see increases, from R to FP of 1.3 and 1.7 log cfu/g, respectively (Figure 2).

In contrast, our data from NFDM shows that for the beginning and middle TSC time points (Figure 3), where there is a concentration factor from raw to finished product of 10:1, the finished product results appear to be primarily attributed to the physical concentration of the raw skim milk as 1.6 log cfu/mL in raw resulted in 2.6 log cfu/g at the beginning time point and 1.3 log cfu/mL in raw resulted in 2.3 log cfu/g at the middle time point (Figure 3). However, at the end timepoint, we see an increase from 1.5 log cfu/mL in raw material to 3.3 log cfu/g in FP (Figure 3). This 1.8 log cfu/g increase exceeds the one-log increase that would be predicted, which leads us to believe that there may indeed be an increase in microbial load over the course of a production run.

These examples are simply intended as exercises in theory – to demonstrate additional considerations that may be contributing to the perceived increase in spore load, from raw to finished product. Additional work (e.g., determination of the ecology of the organisms that we isolated) must be performed to determine the exact contribution from each mechanism, (e.g., the effect of concentration, contamination from the processing environment, or microbial growth and biofilm development). Also, further investigation is warranted to determine potential

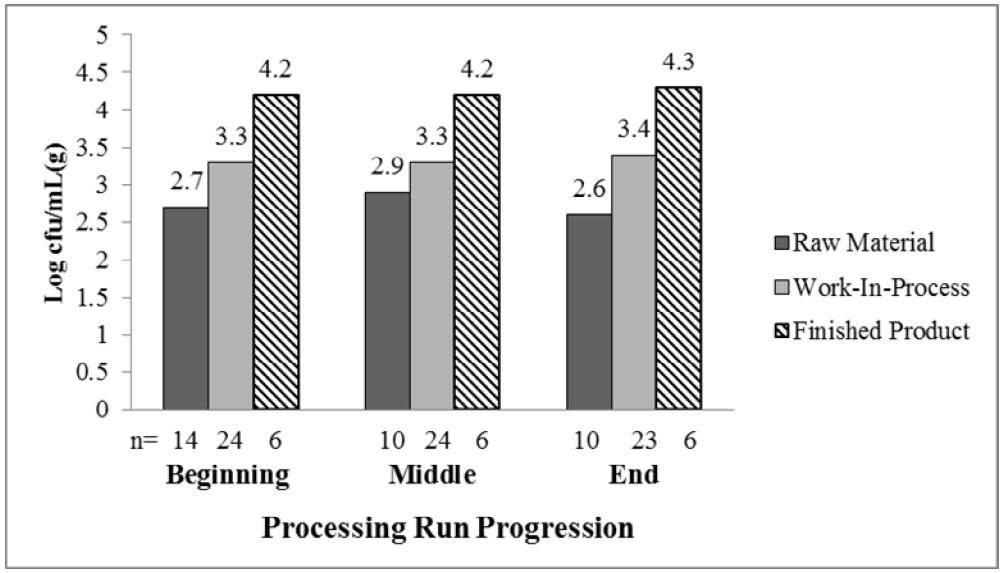

Figure 2. Mean Thermophilic Spore Count (TSC) in log cfu/mL or gram for **Plant A (sweet whey)** samples that were positive on direct plating, by product status: Raw Material, Work-In-Process and Finished Product; and through the course of the production run at three time points: Beginning, Middle and End. The sample size for each category is indicated directly below each status bar and the number above each bar represents the mean value (TSC), as described above.

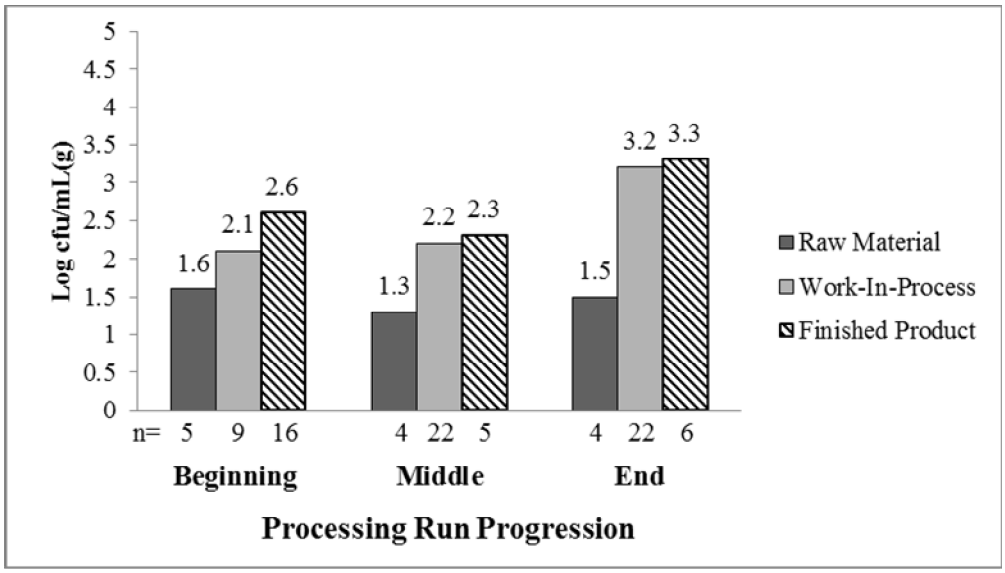

Figure 3. Mean Thermophilic Spore Count (TSC) in log cfu/mL or gram for **Plant B (nonfat dry milk)** samples that were positive on direct plating, by product status: Raw Material, Work-In-Process and Finished Product; and through the course of the production run at three time points: Beginning, Middle and End. The sample size for each category is indicated directly below each status bar and the number above each bar represents the mean value (TSC), as described above.

points of entry, and to detect multiplication of vegetative cells that may be sporulating during the process, and forming biofilms.

Unexpectedly, our results do not indicate that there was a significant increase in the incidence of MSC, TSC, HHR MSC or HHR TSC (p-values ranging from 0.07 to 0.74), throughout the processing run (from beginning to end) in any of the four products tested (statistical analyses not shown). Previous work (Scott et al., 2007) found substantial increases in spore load (>3.0 log cfu/g) throughout a processing run, with the plate heat exchanger identified as the primary location of in-plant spore contamination. Scott et al. (2007) implicated the buildup and sloughing-off of foulant, or the accumulation of layers of burnt milk during a processing run, as potential sources of contamination, and they identified high levels of *Geobacillus* spp. in the foulant samples. Despite the absence of a statistically significant increase in our spore count results (from the beginning to end of the NFDM process), there does appear to be an increase in TSC at the end time point in the WIP and FP samples (Figure 3). Whereas raw material NFDM TSC remains constant over time (B,M, and E), with no change in the order of magnitude in WIP and FP at the B and M timepoints, it is important to note that there is approximately a one-log increase in WIP and FP at the E time point (Figure 3); this phenomenon remains unexplained.

Our data may suggest, that in three of the plants in this study (SWP, AWP and WPC-80), there is little foulant slough-off, or perhaps that slough-off occurs intermittently during the processing run, which may explain the lack of increase in bacterial load during the processing run (e.g., at the M timepoint). There is, however, some evidence in the NFDM plant results for TSC, that indicate that biofilm slough-off or other bacterial contamination may indeed contribute to the increase spore load near the end of the process. We hope to supplement our understanding of this data upon careful examination of the bacterial isolates collected during this study.

CONCLUSIONS

Our results indicate that thermophilic sporeformers are the primary organisms of concern in dairy powders. Thermophilic sporeformers were more prevalent than both mesophilic and psychrotolerant sporeformers in all dairy powders tested. Additionally, we found that the incidence of highly heat resistant sporeformers was lower than other SFs tested. Spore counts of WIP and finished products varied greatly by product type; however, we found that sporeformer counts in raw materials remained reasonably constant throughout the run. The products with the highest thermophilic spore counts were NFDM and SWP; these results suggest a specific need to target spore interventions for those products. Stringent specifications for sporeformers in dairy powders, which are being used by many customers, may be a hurdle to expanding the market for NFDM and SWP. As the demand for dairy powders increases globally, understanding the presence and transmission of thermophilic sporeformers is of great importance to the dairy industry.

APPENDIX A

Supplemental Table 1. Summary of psychrotolerant, mesophilic, and thermophilic spore counts and highly heat resistant mesophilic and thermophilic spore counts for dairy powders from 4 northeast dairies sampled 6 times over a 12-month period

Appendix A: Supplemental Table S1

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
A1-1B	R	<6 cfu/g	200 cfu/g	2700 cfu/g	NT	NT
A1-1M	R	NS	NS	NS	NS	NS
A1-1E	R	NS	NS	NS	NS	NS
A1-2B	R	<3 cfu/g	>3 cfu/g → <100 cfu/g	<3 cfu/g	NT	NT
A1-2M	R	NS	NS	NS	NS	NS
A1-2E	R	NS	NS	NS	NS	NS
A1-3B	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	72 cfu/ml	NT	NT
A1-3M	R	<0.03 cfu/ml	<0.03 cfu/ml	270 cfu/ml	NT	NT
A1-3E	R	<0.03 cfu/ml	<0.03 cfu/ml	2200 cfu/ml	NT	NT
A1-4B	R	<0.03 cfu/ml	<0.03 cfu/ml	41 cfu/ml	NT	NT
A1-4M	R	<0.03 cfu/ml	<0.03 cfu/ml	1400 cfu/ml	NT	NT
A1-4E	R	<0.03 cfu/ml	<0.03 cfu/ml	950 cfu/ml	NT	NT
A1-5B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	200 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A1-5M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	3200 cfu/ml	10 cfu/ml	72 cfu/ml
A1-5E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	7600 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	20 cfu/ml
A1-6B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	1700 cfu/ml	<0.03 cfu/ml	10 cfu/ml
A1-6M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml	10 cfu/ml	<0.03 cfu/ml
A1-6E	WIP	NS	NS	NS	NS	NS
A1-7B	WIP	<0.3 cfu/ml	>0.3 cfu/ml → <100 cfu/ml	8500 cfu/ml	LE	>0.3 cfu/ml → <100 cfu/ml
A1-7M	WIP	<0.3 cfu/ml	>0.3 cfu/ml → <100 cfu/ml	510 cfu/ml	>0.3 cfu/ml → <100 cfu/ml	>0.3 cfu/ml → <100 cfu/ml
A1-7E	WIP	<0.3 cfu/ml	>0.3 cfu/ml → <100 cfu/ml	7400 cfu/ml	>0.3 cfu/ml → <100 cfu/ml	10 cfu/ml
A1-8B	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	16000 cfu/g	>3 cfu/g → <100 cfu/g	10 cfu/g
A1-8M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	22000 cfu/g	<3 cfu/g	310 cfu/g
A1-8E	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	22000 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
A1-9B	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	37000 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g
A1-9M	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	16000 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
A1-9E	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	14000 cfu/g	<3 cfu/g	310 cfu/g
A2-3B	R	LE	10 cfu/ml	170 cfu/ml	NT	NT
A2-3M	R	LE	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
A2-3E	R	LE	>0.03 cfu/ml → <10 cfu/ml	20 cfu/ml	NT	NT
A2-4B	R	LE	<0.03 cfu/ml	160 cfu/ml	NT	NT
A2-4M	R	LE	>0.03 cfu/ml → <10 cfu/ml	5900 cfu/ml	NT	NT
A2-4E	R	LE	>0.03 cfu/ml → <10 cfu/ml	290 cfu/ml	NT	NT
A2-5B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	61 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A2-5M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	20000 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml
A2-5E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	19000 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A2-6B	WIP	<0.03 cfu/ml	10 cfu/ml	2700 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A2-6M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	4400 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A2-6E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A2-7B	WIP	<0.06 cfu/ml	41 cfu/ml	12000 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A2-7M	WIP	<0.06 cfu/ml	<0.06 cfu/ml	19000 cfu/ml	<0.03 cfu/ml	20 cfu/ml
A2-7E	WIP	190000 cfu/ml	800000 cfu/ml [9]	43000 cfu/ml	<0.03 cfu/ml	1800 cfu/ml
A2-8B	WIP	5100 cfu/g	140000 cfu/g	13000 cfu/g	<3 cfu/g	4200 cfu/g

Appendix A: Supplemental Table S1 (Continued)

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
A2-8M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	14000 cfu/g	<3 cfu/g	2200 cfu/g
A2-8E	WIP	<3 cfu/g	100 cfu/g	18000 cfu/g	<3 cfu/g	1300 cfu/g
A2-9B	F	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g	20000 cfu/g	<3 cfu/g	2000 cfu/g
A2-9M	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	7900 cfu/g	>3 cfu/g → <100 cfu/g	12000 cfu/g
A2-9E	F	>3 cfu/g → <100 cfu/g	100 cfu/g	24000 cfu/g	<3 cfu/g	6900 cfu/g
A3-1B	R	LE	200 cfu/g	2000 cfu/g	NT	NT
A3-1M	R	NS	NS	NS	NS	NS
A3-1E	R	NS	NS	NS	NS	NS
A3-2B	R	LE	<3 cfu/g	<3 cfu/g	NT	NT
A3-2M	R	NS	NS	NS	NS	NS
A3-2E	R	NS	NS	NS	NS	NS
A3-3B	R	LE	31 cfu/ml	82 cfu/ml	NT	NT
A3-3M	R	LE	10 cfu/ml	10 cfu/ml	NT	NT
A3-3E	R	LE	>0.03 cfu/ml → <10 cfu/ml	20 cfu/ml	NT	NT
A3-4B	R	LE	>0.03 cfu/ml → <10 cfu/ml	3500 cfu/ml	NT	NT
A3-4M	R	LE	>0.03 cfu/ml → <10 cfu/ml	2100 cfu/ml	NT	NT
A3-4E	R	LE	>0.03 cfu/ml → <10 cfu/ml	1200 cfu/ml	NT	NT
A3-5B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	140 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A3-5M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
A3-5E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	770 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A3-6B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	2600 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml
A3-6M	WIP	<0.03 cfu/ml	31 cfu/ml	4000 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml
A3-6E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	2300 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml
A3-7B	WIP	<0.06 cfu/ml	61 cfu/ml	6700 cfu/ml	<0.06 cfu/ml	550 cfu/ml
A3-7M	WIP	<0.06 cfu/ml	41 cfu/ml	3550 cfu/ml	<0.06 cfu/ml	410 cfu/ml
A3-7E	WIP	<0.06 cfu/ml	20 cfu/ml	4100 cfu/ml	20 cfu/ml	680 cfu/ml
A3-8B	WIP	<3 cfu/g	100 cfu/g	11000 cfu/g	>3 cfu/g → <100 cfu/g	5300 cfu/g
A3-8M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	20000 cfu/g	<3 cfu/g	3100 cfu/g
A3-8E	WIP	<3 cfu/g	310 cfu/g	13000 cfu/g	>3 cfu/g → <100 cfu/g	6000 cfu/g
A3-9B	F	<3 cfu/g	100 cfu/g	9700 cfu/g	100 cfu/g	2400 cfu/g
A3-9M	F	<3 cfu/g	100 cfu/g	11000 cfu/g	<3 cfu/g	3300 cfu/g
A3-9E	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	14000 cfu/g	<3 cfu/g	6800 cfu/g
A4-3B	R	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
A4-3M	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
A4-3E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
A4-4B	R	<0.03 cfu/ml	10 cfu/ml	1400 cfu/ml	NT	NT
A4-4M	R	<0.03 cfu/ml	<0.03 cfu/ml	1200 cfu/ml	NT	NT
A4-4E	R	NS	NS	NS	NS	NS
A4-5B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	150 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A4-5M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	300 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A4-5E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	940 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A4-6B	WIP	<0.03 cfu/ml	10 cfu/ml	320 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A4-6M	WIP	<0.03 cfu/ml	10 cfu/ml	270 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
A4-6E	WIP	<0.03 cfu/ml	20 cfu/ml	4100 cfu/ml	<0.03 cfu/ml	240 cfu/ml
A4-7B	WIP	<0.09 cfu/ml	<0.09 cfu/ml	1600 cfu/ml	<0.09 cfu/ml	62 cfu/ml
A4-7M	WIP	<0.09 cfu/ml	31 cfu/ml	1600 cfu/ml	31 cfu/ml	310 cfu/ml
A4-7E	WIP	<0.09 cfu/ml	31 cfu/ml	41000 cfu/ml	<0.09 cfu/ml	3300 cfu/ml
A4-8B	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	34000 cfu/g	<3 cfu/g	<3 cfu/g
A4-8M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	29000 cfu/g	<3 cfu/g	100 cfu/g
A4-8E	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	19000 cfu/g	<3 cfu/g	<3 cfu/g
A4-9B	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	28000 cfu/g	<3 cfu/g	<3 cfu/g
A4-9M	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	20000 cfu/g	<3 cfu/g	<3 cfu/g
A4-9E	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	22000 cfu/g	>3 cfu/g → <100 cfu/g	<3 cfu/g
A5-1B	R	<3 cfu/g	100 cfu/g	920 cfu/g	NT	NT
A5-1M	R	NS	NS	NS	NS	NS
A5-1E	R	NS	NS	NS	NS	NS
A5-2B	R	<3 cfu/g	<3 cfu/g	<3 cfu/g	NT	NT
A5-2M	R	NS	NS	NS	NS	NS
A5-2E	R	NS	NS	NS	NS	NS
A5-3B	R	<0.03 cfu/ml	<0.03 cfu/ml	11000 cfu/ml	NT	NT
A5-3M	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	50000 cfu/ml	NT	NT
A5-3E	R	<0.03 cfu/ml	<0.03 cfu/ml	21000 cfu/ml	NT	NT
A5-4B	R	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	NT	NT
A5-4M	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml	NT	NT
A5-4E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	20 cfu/ml	NT	NT
A5-5B	WIP	<0.03 cfu/ml	20 cfu/ml	16000 cfu/ml	10 cfu/ml	<0.03 cfu/ml
A5-5M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	31000 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A5-5E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	470 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
A5-6B	WIP	<0.03 cfu/ml	10 cfu/ml	490 cfu/ml	<0.03 cfu/ml	61 cfu/ml
A5-6M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	460 cfu/ml	<0.03 cfu/ml	10 cfu/ml
A5-6E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml
A5-7B	WIP	<0.09 cfu/ml	<0.09 cfu/ml	1600 cfu/ml	<0.09 cfu/ml	1400 cfu/ml
A5-7M	WIP	<0.09 cfu/ml	<0.09 cfu/ml	1900 cfu/ml	<0.09 cfu/ml	580 cfu/ml
A5-7E	WIP	<0.09 cfu/ml	31 cfu/ml	1900 cfu/ml	<0.09 cfu/ml	700 cfu/ml
A5-8B	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	12000 cfu/g	>3 cfu/g → <100 cfu/g	200 cfu/g
A5-8M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	9900 cfu/g	>3 cfu/g → <100 cfu/g	200 cfu/g
A5-8E	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	12000 cfu/g	<3 cfu/g	310 cfu/g
A5-9B	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	10000 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
A5-9M	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	9300 cfu/g	>3 cfu/g → <100 cfu/g	100 cfu/g
A5-9E	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	9800 cfu/g	<3 cfu/g	100 cfu/g
A6-3B	R	<0.03 cfu/ml	10 cfu/ml	20 cfu/ml	NT	NT
A6-3M	R	<0.03 cfu/ml	10 cfu/ml	10 cfu/ml	NT	NT
A6-3E	R	10 cfu/ml	10 cfu/ml	20 cfu/ml	NT	NT
A6-4B	R	<0.03 cfu/ml	31 cfu/ml	46000 cfu/ml	NT	NT
A6-4M	R	<0.03 cfu/ml	<0.03 cfu/ml	45000 cfu/ml	NT	NT
A6-4E	R	<0.03 cfu/ml	<0.03 cfu/ml	32000 cfu/ml	NT	NT

Appendix A: Supplemental Table S1 (Continued)

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
A6-5B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	580 cfu/ml	10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
A6-5M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	620 cfu/ml	10 cfu/ml	10 cfu/ml
A6-5E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	410 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
A6-6B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	20 cfu/ml	10 cfu/ml
A6-6M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml	10 cfu/ml	<0.03 cfu/ml
A6-6E	WIP	<0.03 cfu/ml	20 cfu/ml	10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
A6-7B	WIP	<0.09 cfu/ml	<0.09 cfu/ml	1600 cfu/ml	<0.09 cfu/ml	640 cfu/ml
A6-7M	WIP	<0.09 cfu/ml	31 cfu/ml	1400 cfu/ml	<0.09 cfu/ml	220 cfu/ml
A6-7E	WIP	<0.09 cfu/ml	92 cfu/ml	2300 cfu/ml	<0.09 cfu/ml	2700 cfu/ml
A6-8B	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	78000 cfu/g	100 cfu/g	2400 cfu/g
A6-8M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	76000 cfu/g	>3 cfu/g → <100 cfu/g	1000 cfu/g
A6-8E	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	39000 cfu/g	>3 cfu/g → <100 cfu/g	920 cfu/g
A6-9B	F	<3 cfu/g	<3 cfu/g	11000 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
A6-9M	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	94000 cfu/g	<3 cfu/g	1400 cfu/g
A6-9E	F	<3 cfu/g	100 cfu/g	91000 cfu/g	310 cfu/g	1600 cfu/g
B1-1B	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	20 cfu/ml	NT	NT
B1-1M	R	<0.03 cfu/ml	31 cfu/ml	LE	NT	NT
B1-1E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
B1-2B	WIP	<0.03 cfu/ml	10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B1-2M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B1-2E	WIP	>0.03 cfu/ml → <10 cfu/ml	400 cfu/ml	1100 cfu/ml	<0.06 cfu/ml	200 cfu/ml
B1-3B	WIP	>0.3 cfu/ml → <100 cfu/ml	410 cfu/ml	100 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B1-3M	WIP	>0.3 cfu/ml → <100 cfu/ml	100 cfu/ml	220 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B1-3E	WIP	>0.3 cfu/ml → <100 cfu/ml	510 cfu/ml	38000 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	20 cfu/ml
B1-4B	WIP	<0.24 cfu/ml	240 cfu/ml	>0.24 cfu/ml → <80 cfu/ml	<0.06 cfu/ml	20 cfu/ml
B1-4M	WIP	<0.12 cfu/ml	200 cfu/ml	370 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml
B1-4E	WIP	<0.6 cfu/ml	610 cfu/ml	61000 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	20 cfu/ml
B1-5B	WIP	<3 cfu/g	100 cfu/g	62 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g
B1-5M	WIP	>3 cfu/g → <100 cfu/g	200 cfu/g	170 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
B1-5E	WIP	<3 cfu/g	200 cfu/g	10000 cfu/g	>3 cfu/g → <100 cfu/g	410 cfu/g
B1-6B	F	<3 cfu/g	200 cfu/g	100 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g
B1-6M	F	<3 cfu/g	340 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
B1-6E	F	<3 cfu/g	510 cfu/g	860 cfu/g	<3 cfu/g	310 cfu/g
B2-1B	R	<0.03 cfu/ml	61 cfu/ml	51 cfu/ml	NT	NT
B2-1M	R	<0.03 cfu/ml	82 cfu/ml	20 cfu/ml	NT	NT
B2-1E	R	<0.03 cfu/ml	10 cfu/ml	10 cfu/ml	NT	NT
B2-2B	WIP	<0.03 cfu/ml	2600 cfu/ml	92 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
B2-2M	WIP	<0.03 cfu/ml	41 cfu/ml	41 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B2-2E	WIP	>0.03 cfu/ml → <10 cfu/ml	1200 cfu/ml	20 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B2-3B	WIP	<0.03 cfu/ml	72 cfu/ml	540 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B2-3M	WIP	<0.03 cfu/ml	51 cfu/ml	550 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	20 cfu/ml
B2-3E	WIP	>0.03 cfu/ml → <10 cfu/ml	61 cfu/ml	1200 cfu/ml	<0.06 cfu/ml	41 cfu/ml
B2-4B	WIP	<0.06 cfu/ml	290 cfu/ml	240 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	20 cfu/ml

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
B2-4M	WIP	<0.06 cfu/ml	82 cfu/ml	440 cfu/ml	<0.06 cfu/ml	20 cfu/ml
B2-4E	WIP	<0.06 cfu/ml	82 cfu/ml	92 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	61 cfu/ml
B2-5B	WIP	<3 cfu/g	1000 cfu/g	61 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g
B2-5M	WIP	>3 cfu/g → <100 cfu/g	720 cfu/g	62 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
B2-5E	WIP	<3 cfu/g	200 cfu/g	360 cfu/g	>3 cfu/g → <100 cfu/g	610 cfu/g
B2-6B	F	<3 cfu/g	200 cfu/g	72 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
B2-6M	F	<3 cfu/g	100 cfu/g	82 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g
B2-6E	F	<3 cfu/g	410 cfu/g	120 cfu/g	>3 cfu/g → <100 cfu/g	200 cfu/g
B3-1B	R	<0.03 cfu/ml	51 cfu/ml	31 cfu/ml	NT	NT
B3-1M	R	<0.03 cfu/ml	82 cfu/ml	20 cfu/ml	NT	NT
B3-1E	R	<0.03 cfu/ml	61 cfu/ml	130 cfu/ml	NT	NT
B3-2B	WIP	<0.03 cfu/ml	20 cfu/ml	41 cfu/ml	320 cfu/ml	10 cfu/ml
B3-2M	WIP	<0.03 cfu/ml	10 cfu/ml	92 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	61 cfu/ml
B3-2E	WIP	NS	NS	NS	NS	NS
B3-3B	WIP	<0.03 cfu/ml	31 cfu/ml	51 cfu/ml	1900 cfu/ml	160 cfu/ml
B3-3M	WIP	<0.03 cfu/ml	82 cfu/ml	140 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B3-3E	WIP	<0.03 cfu/ml	41 cfu/ml	720 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	510 cfu/ml
B3-4B	WIP	<0.06 cfu/ml	120 cfu/ml	630 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	610 cfu/ml
B3-4M	WIP	<0.06 cfu/ml	120 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
B3-4E	WIP	<0.06 cfu/ml	82 cfu/ml	120 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
B3-5B	WIP	<3 cfu/g	610 cfu/g	1000 cfu/g	>3 cfu/g → <100 cfu/g	100 cfu/g
B3-5M	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	820 cfu/g	>3 cfu/g → <100 cfu/g	200 cfu/g
B3-5E	WIP	<3 cfu/g	610 cfu/g	4600 cfu/g	>3 cfu/g → <100 cfu/g	820 cfu/g
B3-6B	F	<3 cfu/g	310 cfu/g	1900 cfu/g	>3 cfu/g → <100 cfu/g	100 cfu/g
B3-6M	F	<3 cfu/g	200 cfu/g	410 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
B3-6E	F	<3 cfu/g	310 cfu/g	1100 cfu/g	>3 cfu/g → <100 cfu/g	1100 cfu/g
B4-1B	R	<0.03 cfu/ml	51 cfu/ml	110 cfu/ml	NT	NT
B4-1M	R	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml	20 cfu/ml	NT	NT
B4-1E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	51 cfu/ml	NT	NT
B4-2B	WIP	<0.03 cfu/ml	20 cfu/ml	20 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B4-2M	WIP	<0.03 cfu/ml	51 cfu/ml	92 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B4-2E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	72 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B4-3B	WIP	<0.03 cfu/ml	72 cfu/ml	100 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	20 cfu/ml
B4-3M	WIP	<0.03 cfu/ml	82 cfu/ml	460 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	700 cfu/ml
B4-3E	WIP	<0.03 cfu/ml	20 cfu/ml	39000 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	86000 cfu/ml
B4-4B	WIP	<0.06 cfu/ml	180 cfu/ml	310 cfu/ml	20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B4-4M	WIP	<0.06 cfu/ml	120 cfu/ml	470 cfu/ml	20 cfu/ml	52000 cfu/ml
B4-4E	WIP	<0.06 cfu/ml	20 cfu/ml	45000 cfu/ml	20 cfu/ml	100 cfu/ml
B4-5B	WIP	<3 cfu/g	20 cfu/g	130 cfu/g	20 cfu/g	>3 cfu/g → <100 cfu/g
B4-5M	WIP	<3 cfu/g	41 cfu/g	120 cfu/g	>3 cfu/g → <100 cfu/g	41 cfu/g
B4-5E	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	14000 cfu/g	>3 cfu/g → <100 cfu/g	26000 cfu/g
B4-6B	F	<3 cfu/g	41 cfu/g	190 cfu/g	>3 cfu/g → <100 cfu/g	10 cfu/g
B4-6M	F	<3 cfu/g	51 cfu/g	100 cfu/g	>3 cfu/g → <100 cfu/g	61 cfu/g

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
B4-6E	F	<3 cfu/g	10 cfu/g	21000 cfu/g	>3 cfu/g → <100 cfu/g	20000 cfu/g
B5-1B	R	<0.03 cfu/ml	LE	>0.03 cfu/ml → <10 cfu/ml	NT	NT
B5-1M	R	<0.03 cfu/ml	31 cfu/ml	20 cfu/ml	NT	NT
B5-1E	R	<0.03 cfu/ml	20 cfu/ml	LE	NT	NT
B5-2B	WIP	<0.03 cfu/ml	10 cfu/ml	10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B5-2M	WIP	<0.03 cfu/ml	31 cfu/ml	62 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B5-2E	WIP	<0.03 cfu/ml	31 cfu/ml	180 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B5-3B	WIP	<0.03 cfu/ml	20 cfu/ml	82 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B5-3M	WIP	<0.03 cfu/ml	41 cfu/ml	450 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	590 cfu/ml
B5-3E	WIP	<0.03 cfu/ml	72 cfu/ml	92 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	510 cfu/ml
B5-4B	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B5-4M	WIP	<0.09 cfu/ml	62 cfu/ml	520 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	82 cfu/ml
B5-4E	WIP	<0.09 cfu/ml	120 cfu/ml	1400 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	62 cfu/ml
B5-5B	WIP	<3 cfu/g	10 cfu/g	410 cfu/g	200 cfu/g	>3 cfu/g → <100 cfu/g
B5-5M	WIP	<3 cfu/g	200 cfu/g	1600 cfu/g	>3 cfu/g → <100 cfu/g	100 cfu/g
B5-5E	WIP	<3 cfu/g	>3 cfu/g → <100 cfu/g	720 cfu/g	>3 cfu/g → <100 cfu/g	820 cfu/g
B5-6B	F	<3 cfu/g	100 cfu/g	1500 cfu/g	>3 cfu/g → <100 cfu/g	920 cfu/g
B5-6M	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	1400 cfu/g	>3 cfu/g → <100 cfu/g	100 cfu/g
B5-6E	F	<3 cfu/g	200 cfu/g	1500 cfu/g	>3 cfu/g → <100 cfu/g	720 cfu/g
B6-1B	R	<0.03 cfu/ml	20 cfu/ml	51 cfu/ml	NT	NT
B6-1M	R	<0.03 cfu/ml	10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
B6-1E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml	NT	NT
B6-2B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	82 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B6-2M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
B6-2E	WIP	<0.03 cfu/ml	41 cfu/ml	160 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	31 cfu/ml
B6-3B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B6-3M	WIP	<0.03 cfu/ml	20 cfu/ml	82 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
B6-3E	WIP	<0.03 cfu/ml	51 cfu/ml	8800 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	2400 cfu/ml
B6-4B	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
B6-4M	WIP	<0.09 cfu/ml	120 cfu/ml	240 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
B6-4E	WIP	<0.09 cfu/ml	31 cfu/ml	8300 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	150 cfu/ml
B6-5B	WIP	<3 cfu/g	100 cfu/g	2700 cfu/g	<3 cfu/g	720 cfu/g
B6-5M	WIP	<3 cfu/g	100 cfu/g	10 cfu/g	>3 cfu/g → <100 cfu/g	>3 cfu/g → <100 cfu/g
B6-5E	WIP	NS	NS	NS	NS	NS
B6-6B	F	<3 cfu/g	100 cfu/g	720 cfu/g	>3 cfu/g → <100 cfu/g	100 cfu/g
B6-6M	F	<3 cfu/g	310 cfu/g	100 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g
B6-6E	F	<3 cfu/g	310 cfu/g	15000 cfu/g	<3 cfu/g	3700 cfu/g
C1-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C1-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C1-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C1-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C1-2M	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C1-2E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
C1-3B	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C1-3M	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C1-3E	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C1-4B	F	<3 cfu/g	>3 cfu/g → <100 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C1-4M	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C1-4E	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C2-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C2-1M	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	NT	NT
C2-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C2-2B	WIP	<0.06 cfu/ml	61 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C2-2M	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
C2-2E	WIP	<0.06 cfu/ml	41 cfu/ml	61 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C2-3B	WIP	10 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C2-3M	WIP	<0.06 cfu/ml	41 cfu/ml	41 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C2-3E	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml
C2-4B	F	480 cfu/g	>3 cfu/g → <100 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C2-4M	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C2-4E	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C3-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C3-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C3-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C3-2B	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C3-2M	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
C3-2E	WIP	<0.06 cfu/ml	20 cfu/ml	20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C3-3B	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C3-3M	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C3-3E	WIP	<0.06 cfu/ml	<0.06 cfu/ml	20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C3-4B	F	<3 cfu/g	<3 cfu/g	100 cfu/g	<3 cfu/g	<3 cfu/g
C3-4M	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C3-4E	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C4-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	NT	NT
C4-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C4-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C4-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C4-2M	WIP	<0.03 cfu/ml	10 cfu/ml	<0.03 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C4-2E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C4-3B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C4-3M	WIP	<0.03 cfu/ml	10 cfu/ml	72 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C4-3E	WIP	<0.03 cfu/ml	31 cfu/ml	<0.03 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C4-4B	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C4-4M	F	<3 cfu/g	<3 cfu/g	31 cfu/g	<3 cfu/g	<3 cfu/g
C4-4E	F	<3 cfu/g	<3 cfu/g	10 cfu/g	<3 cfu/g	<3 cfu/g
C5-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT

Appendix A: Supplemental Table S1 (Continued)

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
C5-1M	R	<0.03 cfu/ml	20 cfu/ml	<0.03 cfu/ml	NT	NT
C5-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C5-2B	WIP	<0.03 cfu/ml	31 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C5-2M	WIP	<0.03 cfu/ml	20 cfu/ml	41 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C5-2E	WIP	<0.03 cfu/ml	20 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C5-3B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C5-3M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	31 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C5-3E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
C5-4B	F	<3 cfu/g	<3 cfu/g	410 cfu/g	<3 cfu/g	<3 cfu/g
C5-4M	F	<3 cfu/g	100 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C5-4E	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C6-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C6-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C6-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
C6-2B	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C6-2M	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C6-2E	WIP	<0.06 cfu/ml	20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C6-3B	WIP	<0.06 cfu/ml	20 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml
C6-3M	WIP	<0.06 cfu/ml	20 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C6-3E	WIP	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml	<0.06 cfu/ml
C6-4B	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C6-4M	F	<3 cfu/g	20 cfu/g	<3 cfu/g	<3 cfu/g	<3 cfu/g
C6-4E	F	<3 cfu/g	<3 cfu/g	<3 cfu/g	>3 cfu/g → <100 cfu/g	<3 cfu/g
D1-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D1-1M	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	10 cfu/ml	NT	NT
D1-1E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	NT	NT
D1-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D1-2M	WIP	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	130 cfu/ml	<0.03 cfu/ml
D1-2E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D1-3B	WIP	<0.03 cfu/ml	10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D1-3M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D1-3E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D1-4B	WIP	<0.15 cfu/ml	>0.15 cfu/ml → <50 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml
D1-4M	WIP	<0.15 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml	>0.15 cfu/ml → <50 cfu/ml
D1-4E	WIP	<0.15 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml	100 cfu/ml	<0.15 cfu/ml
D1-5B	WIP	NS	NS	NS	NS	NS
D1-5M	WIP	NS	NS	NS	NS	NS
D1-5E	WIP	<9 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	<15 cfu/g	<15 cfu/g
D1-6B	F	NS	NS	NS	NS	NS
D1-6M	F	NS	NS	NS	NS	NS
D1-6E	F	<9 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	>15 cfu/g → <500 cfu/g	<15 cfu/g
D1-7B	R	<0.15 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml	NT	NT
D1-7M	R	NS	NS	NS	NS	NS

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
D1-7E	R	NS	NS	NS	NS	NS
D2-1B	R	41000 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D2-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D2-1E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
D2-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D2-2M	WIP	<0.03 cfu/ml	2100 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D2-2E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	520 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D2-3B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	51 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D2-3M	WIP	92 cfu/ml	10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
D2-3E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml
D2-4B	WIP	<0.15 cfu/ml	>0.15 cfu/ml → <50 cfu/ml	51 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml
D2-4M	WIP	<0.15 cfu/ml	>0.15 cfu/ml → <50 cfu/ml	<0.15 cfu/ml	>0.15 cfu/ml → <50 cfu/ml	<0.15 cfu/ml
D2-4E	WIP	<0.15 cfu/ml	<0.15 cfu/ml	<0.15 cfu/ml	>0.15 cfu/ml → <50 cfu/ml	<0.15 cfu/ml
D2-5B	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D2-5M	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D2-5E	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D2-6B	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D2-6M	F	<6 cfu/g	200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D2-6E	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D3-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D3-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D3-1E	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D3-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	<0.03 cfu/ml
D3-2M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D3-2E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D3-3B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D3-3M	WIP	<0.03 cfu/ml	LE	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D3-3E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D3-4B	WIP	<0.12 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml
D3-4M	WIP	<0.12 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml
D3-4E	WIP	<0.12 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml	>0.12 cfu/ml → <40 cfu/ml
D3-5B	WIP	<6 cfu/g	200 cfu/g	200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D3-5M	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D3-5E	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	66000 cfu/g	>6 cfu/g → <200 cfu/g
D3-6B	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g
D3-6M	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D3-6E	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	620 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D4-1B	R	<0.03 cfu/ml	51 cfu/ml	10 cfu/ml	NT	NT
D4-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	NT	NT
D4-1E	R	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	NT	NT
D4-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D4-2M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D4-2E	WIP	<0.03 cfu/ml	640 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
D4-3B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
D4-3M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
D4-3E	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
D4-4B	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
D4-4M	WIP	<0.09 cfu/ml	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
D4-4E	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
D4-5B	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	410 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D4-5M	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D4-5E	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D4-6B	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D4-6M	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g
D4-6E	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D4-7B	R	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	<0.09 cfu/ml	NT	NT
D4-7M	R	NS	NS	NS	NS	NS
D4-7E	R	NS	NS	NS	NS	NS
D5-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D5-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	NT	NT
D5-1E	R	<0.03 cfu/ml	10 cfu/ml	<0.03 cfu/ml	NT	NT
D5-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D5-2M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D5-2E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D5-3B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D5-3M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D5-3E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D5-4B	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
D5-4M	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
D5-4E	WIP	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	>0.09 cfu/ml → <30 cfu/ml	<0.09 cfu/ml	>0.09 cfu/ml → <30 cfu/ml
D5-5B	WIP	<9 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g
D5-5M	WIP	<9 cfu/g	>9 cfu/g → <300 cfu/g	310 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g
D5-5E	WIP	<9 cfu/g	310 cfu/g	>9 cfu/g → <300 cfu/g	<9 cfu/g	>9 cfu/g → <300 cfu/g
D5-6B	F	<9 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	<9 cfu/g	>9 cfu/g → <300 cfu/g
D5-6M	F	<9 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	<9 cfu/g	>9 cfu/g → <300 cfu/g
D5-6E	F	<9 cfu/g	>9 cfu/g → <300 cfu/g	>9 cfu/g → <300 cfu/g	<9 cfu/g	>9 cfu/g → <300 cfu/g
D5-7B	R	NS	NS	NS	NS	NS
D5-7M	R	NS	NS	NS	NS	NS
D5-7E	R	NS	NS	NS	NS	NS
D6-1B	R	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	NT	NT
D6-1M	R	<0.03 cfu/ml	<0.03 cfu/ml	31 cfu/ml	NT	NT
D6-1E	R	<0.03 cfu/ml	31 cfu/ml	<0.03 cfu/ml	NT	NT
D6-2B	WIP	<0.03 cfu/ml	<0.03 cfu/ml	10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D6-2M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D6-2E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D6-3B	WIP	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml

Sample ID[1,2,3,4,5]	Status[6]	PSC[7,8,9]	MSC[7]	TSC[7]	HHR MSC[10, 11, 12]	HHR TSC[10]
D6-3M	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml
D6-3E	WIP	<0.03 cfu/ml	<0.03 cfu/ml	>0.03 cfu/ml → <10 cfu/ml	<0.03 cfu/ml	<0.03 cfu/ml
D6-4B	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
D6-4M	WIP	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	20 cfu/ml	<0.06 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
D6-4E	WIP	<0.06 cfu/ml	20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml	>0.06 cfu/ml → <20 cfu/ml
D6-5B	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	410 cfu/g
D6-5M	WIP	<6 cfu/g	>6 cfu/g → <200 cfu/g	200 cfu/g	<6 cfu/g	200 cfu/g
D6-5E	WIP	<6 cfu/g	200 cfu/g	200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D6-6B	F	<6 cfu/g	410 cfu/g	>6 cfu/g → <200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D6-6M	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D6-6E	F	<6 cfu/g	>6 cfu/g → <200 cfu/g	200 cfu/g	<6 cfu/g	>6 cfu/g → <200 cfu/g
D6-7B	R	NS	NS	NS	NS	NS
D6-7M	R	NS	NS	NS	NS	NS
D6-7E	R	NS	NS	NS	NS	NS

[1]Sample ID code: "A1-2B" = Plant A; Sample Cycle 1; Sample Point 2; Beginning Time point

[2]Sample ID code: "B2-3M" = Plant B; Sample Cycle 2; Sample Point 3; Middle Time point

[3]Sample ID code: "C3-4E" = Plant C; Sample Cycle 3; Sample Point 4; End Time point

[4]Sample ID code: "D4-5B" = Plant D; Sample Cycle 4; Sample Point 5; Beginning Time point (Sample Cycle numbering convention continues up to 6 and Sample Points numbering continues up to 9)

[5]B = Beginning (sampled within 2h of run start); M = Middle (sampled within 2h of run midpoint); E = End (sampled 2h of run finish)

[6]R = Raw; WIP = Work in process; F = Finished product

[7]PSC = Psychrotolerant Spore Count; MSC = Mesophilic Spore Count; TSC = Thermophilic Spore Count

[8]All values <10 cfu/ml or g and >400,000 cfu/ml or g are estimated values (normally identified by "E")

[9]NS = No sample submitted

[10]HHR MSC = Highly Heat Resistant Mesophilic Spore Count; HHR TSC = Highly Heat Resistant Thermophilic Spore Count

[11]NT = Not tested

[12]LE = Lab error

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